Bone 81 (2015) 247–254

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Original Full Length Article

Altered material properties are responsible for bone fragility in rats with chronic kidney injury☆

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ARTICLE INFO

Article history:
Received 16 February 2015
Revised 2 July 2015
Accepted 13 July 2015
Available online 15 July 2015

Keywords:
Chronic kidney injury
Pentosidine
Biological apatite
Elastic bone mechanical property

ABSTRACT

Chronic kidney disease (CKD) is associated with an increased risk of fragility fractures, but the underlying pathophysiological mechanism remains obscure. We performed an in vivo experimental study to examine the roles of uremia and abnormal mineral/parathyroid metabolism in the development of bone metabolic abnormalities in uremic rats. Male Sprague-Dawley rats were divided into four groups, comprising sham operation (high turnover bone control = HTB-Cont), 5/6-nephrectomy (high turnover bone nephrectomized = HTB-Nx), thyroparathyroidectomy (low turnover bone control = LTB-Cont), and thyroparathyroidectomy plus 5/6 nephrectomy (low turnover bone nephrectomized = LTB-Nx), and maintained for 16 weeks. Uremia was successfully created in the LTB-Nx and HTB-Nx groups, while hyperparathyroidism was only found in the HTB-Nx group. Cancellous bone histomorphometry revealed significantly higher bone turnover in the HTB-Nx group than in the LTB-Nx group. Storage modulus at 1 Hz and tan delta in cortical bone of the femur, which represent the viscoelastic mechanical properties, were significantly lower in both Nx groups than in the Cont groups regardless of bone metabolism. Pentosidine-to-matrix ratio was increased and crystallinity was decreased in both Nx groups regardless of bone turnover. Mineral-to-matrix ratio was significantly decreased in the HTB-Nx group, but increased in the LTB-Nx group. Enzymatic collagen crosslinks were decreased in the HTB-Nx group. The degree of orientation of the c-axis in carbonated hydroxyapatite (biological apatite = BAp) crystallites was decreased in both Nx groups regardless of bone metabolism. Stepwise multivariate regression revealed that pentosodine-to-matrix ratio and BAp preferential c-axis orientation were significantly associated with storage modulus and tan delta. In conclusion, bone elastic mechanical properties deteriorated regardless of bone metabolism or bone mass in rats with chronic kidney injury. Various changes in bone mineral properties were associated with CKD, including abnormal parathyroid function, impaired bone turnover, and uremia associated with the accumulation of uremic toxins, were responsible for these changes. Pentosidine-to-matrix ratio and BAp orientation at position 5 were the two meaningful determinants of elastic bone mechanical strength, and both factors were associated with the severity of uremia, but not parathyroid function or bone metabolism. These two factors may account for the increased bone fragility among CKD patients.

1. Introduction

Chronic kidney disease (CKD) is associated with abnormalities in systemic mineral metabolism, which is termed CKD-related mineral and bone disease (CKD-MBD) [1]. Abnormal bone metabolism is one of the major manifestations of CKD-MBD. In fact, abnormal bone turnover and/or bone mineralization derived from abnormal systemic mineral metabolism are commonly found among CKD patients [2,3].

CKD is also associated with an increased risk of fragility fractures. Predialysis patients with an estimated glomerular filtration rate of <45 ml/min/1.73 m^2 have twice the risk of hip fractures compared with healthy individuals [4]. Moreover, dialysis patients have a several times higher risk of hip fractures than the general population [5,6].

Bone mass is a partial predictor of fracture [7], even among CKD patients [8,9]. The determinants of bone strength other than bone mass are collectively referred to as bone quality. Bone quality relies on many factors including bone turnover, mineralization, microarchitecture, collagen crosslinking, and matrix composition [7].

Abnormalities in systemic mineral metabolism could alter both bone mass and bone quality in CKD patients. In particular, abnormal bone turnover and bone mineralization are predominantly caused by abnormal parathyroid and/or mineral metabolisms in this disease

http://dx.doi.org/10.1016/j.bone.2015.07.015
8756-3282/

condition [10]. Thus, CKD-MBD may account for the increased risk of fragility fractures among CKD patients, at least in part.

However, reduced bone mass and abnormal systemic mineral metabolism, namely CKD-MBD, may not be the only cause of the increased bone fragility in CKD patients. This is because the material properties, including the chemical composition, relative amounts, and distributions of mineral and matrix, govern the mechanical properties of bone [11]. We previously reported that the mineral and matrix composition in the femoral diaphysis showed specific alterations in uremic rats without secondary hyperparathyroidism, and those material changes were tightly associated with bone mechanical deterioration [12,13]. Moreover, such alterations in bone material composition and mechanical properties were at least partially rescued by administration of an oral adsorbent that reduced the circulating uremic toxin levels [13]. Thus, we hypothesized that the accumulation of uremic toxins is another possible candidate for the increased bone fragility.

Nevertheless, we did not intend to negate the role of CKD-MBD in the increased bone fragility in the CKD condition. Both abnormal systemic mineral metabolism and uremic toxins are possible candidates for the pathogenesis of the increased bone fragility in the uremic condition. Thus, we performed an in vivo experimental study using rats with chronic kidney injury and various degrees of parathyroid function. The aim of this study was to evaluate the roles of uremia and abnormal mineral/parathyroid metabolism in the development of bone abnormalities in the uremic condition.

2. Materials and methods

All experiments, including animal handling and testing, were approved by the Animal Care and Use Committee of the Biomedical Research Laboratories, Kureha Chemical Industry Co. Ltd., Japan.

2.1. Animal experiments

A rat model of uremia with high-turnover bone (HTB) or low-turnover bone (LTB) was established using a surgical procedure. The animal experiment protocol is outlined in Fig. 1. All rats were allowed to acclimate in an animal facility for 1 week at constant room temperature with a 12-h/12-h light/dark cycle. To create a rat model of HTB, 12-week-old male Sprague-Dawley (SD) rats were randomly assigned to surgical 5/6 nephrectomy (Nx) (HTB-Nx group) or sham operation (HTB-Cont group). At 1-week intervals, the entire right kidney was removed along with the upper and lower thirds of the left kidney for the 5/6 Nx operations. The remaining rats underwent thyroparathyroidectomy (TPTx) combined with Nx (LTB-Nx group) or TPTx only (LTB-Cont) to make a rat model of uremia with LTB as previously described [14]. All rats that underwent TPTx with or without Nx received continuous infusion of rat 1-34 PTH (Peninsula Laboratories, San Carlos, CA) at a dose of 0.1 μg/kg/h using a subcutaneously implanted Alzet osmotic minipump (Model 2002; Alza Corp., Palo Alto, CA) that was replaced every 2 weeks. To treat the hypothyroid condition associated with TPTx, L-thyroxin (Sigma Chemical Company, St. Louis, MO) was subcutaneously injected at a dose of 4 μg/kg body weight three times per week starting at 2 days after TPTx. At designated time points shown in Fig. 1, 24-h urine samples were collected from each rat using metabolic cages, following which rats were killed under ether anesthesia. Blood samples were collected from the abdominal aorta for serum measurements. Six animals were euthanized at the first operation to collect baseline data. The remaining animals were euthanized at 16 weeks and their right tibias were used for bone histomorphometric analyses. The right femur was used for bone mineral density (BMD), mechanical property, chemical composition, and microbeam transmission X-ray diffraction (μXRD) analyses.

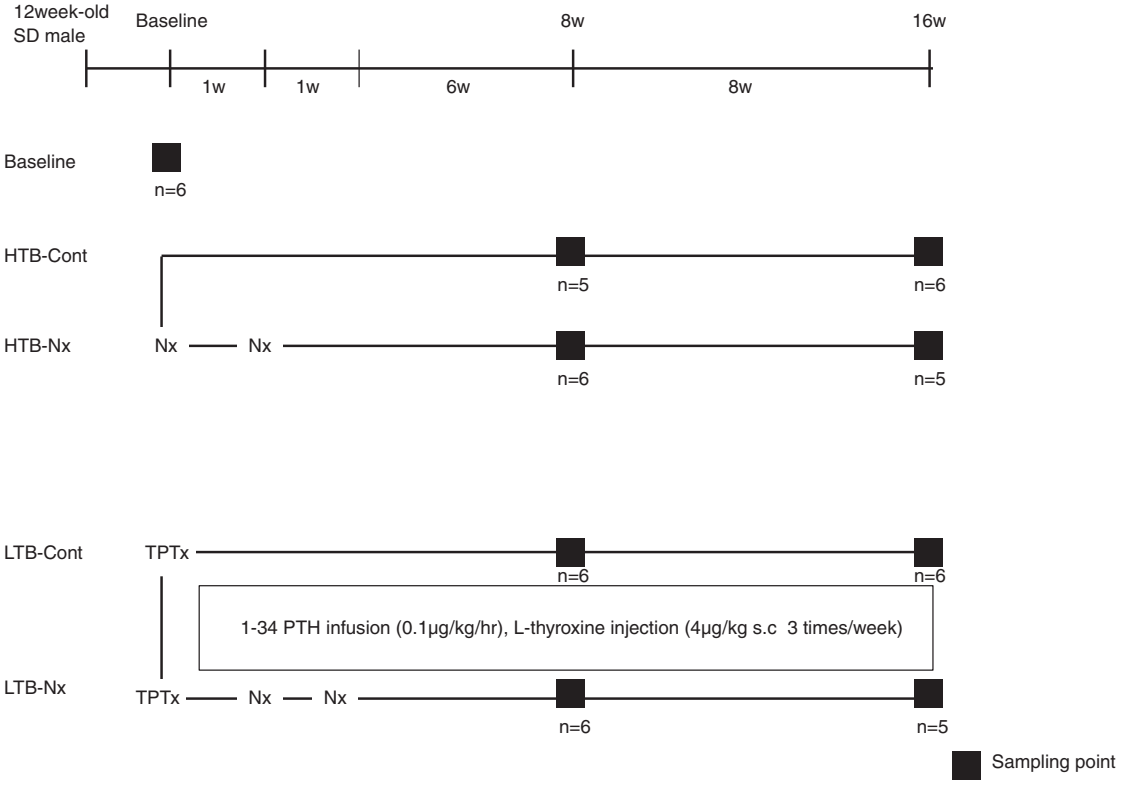

Fig. 1. Experimental protocol for producing high-turnover and low-turnover bone disease with a rat model of chronic kidney injury. HTB-Nx: chronic kidney injury rats with high-turnover bone; HTB-Cont: sham-operated rats with normal kidney function and normal bone turnover; LTB-Nx: chronic kidney injury rats with low-turnover bone; LTB-Cont: rats that underwent TPTx and had normal kidney function with normal bone turnover. TPTx: thyroparathyroidectomy; Nx: nephrectomy. The model rats were created by a surgical procedure. The details are described in the Materials and methods.